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Evaluation of Digestible Energy and Protein for Growth and Nitrogen Retention in Juvenile Florida Pompano, *Trachinotus carolinus*

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Abstract

Juvenile Florida pompano (6.3 ± 0.50 g) were fed 1 of 12 diets formulated with an array of crude protein (340, 380, 420, 480, or 500 g/kg diet) and crude lipid (60, 100, 120, 160, or 180 g/kg diet) levels and estimated digestible protein to digestible energy (DP/DE) of 18.9–26.8 mg/kJ. In a second trial, apparent protein and energy digestibilities were empirically determined and coefficients used to calculate actual digestibilities. Digestible energy (DE) intake was 4.2–13.0 kJ/fish/d, and digestible protein (DP) intake was 0.13–0.32 g/fish/d. Average daily gain increased as a function of both DP and energy. Growth increased with increasing DP in all diets containing 24.0 mg/kJ DP/DE or greater until a plateau at 366 g DP/kg. Nitrogen gain was also a function of both DP and DE. Increasing energy at constant protein improved protein utilization. DP to maximize growth and nitrogen gain was between 356 and 366 g/kg. DE to attain maximum growth in juvenile Florida pompano is at least 15.4 MJ/kg with a DP/DE between 23.8 and 25.1 mg/kJ.

Florida pompano, *Trachinotus carolinus*, represents a small marine fishery in Florida with an estimated 227,000 kg total annual catch; however, because of its highly prized taste and texture, it continues to maintain a high market demand (McMaster et al. 2004). Florida pompano tolerate a wide range of salinities (Allen and Avault 1970), are resistant to low dissolved oxygen and handling stress, readily consume pelleted rations, successfully breed in captivity (Weirich and Riley 2007), and are an excellent candidate for aquaculture in a variety of systems (McMaster et al. 2004). However, little is known about the nutrient requirements of Florida pompano.

Growth and efficiency of Florida pompano fed diets formulated for other species, typically trout feed with a 40% crude protein (CP), resulted in good growth and survival but poor feed efficiency (FE) (Watanabe 1995). As with many other marine species, Florida pompano are presumed to require high dietary CP. Lazo et al. (1998) feeding juvenile Florida pompano four isoenergetic diets (16.75 MJ/kg diet) with graded levels of CP determined that the minimum requirement was 45% CP; however, this was the highest concentration of CP fed.

Lower FE reported for Florida pompano than some other marine species has been attributed to a high metabolic rate and poor digestibility (Tatum 1973; McMaster 1988; Lazo et al. 1998). Florida pompano are highly active, which suggests that previous diets may have had insufficient digestible energy (DE) to support metabolic and growth demands. When juvenile Florida pompano were fed a diet (53% CP and 13% crude lipid [CL]) at various feeding frequencies, FE was better than previously reported for Florida pompano; however, based on proximate analysis, Weirich et al. (2006) suggested that dietary energy was too high. Williams et al. (1985) fed juvenile Florida pompano four isonitrogenous diets formulated with 0–12% menhaden oil and determined that the optimum level of fish oil in a 42% CP diet was between 4 and 8%, providing DE between 10.5 and 11.7 kJ/g diet and a digestible protein to digestible energy (DP/DE) ratio between 29.4 and 32.3 mg DP/kJ DE.

Identification of protein and energy requirements and determination of nutrient digestibility have reduced dietary CP requirements in some

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species due in part to the protein-sparing action of other dietary energy sources (Cho and Bureau 2001). Moreover, protein sparing was shown to increase nitrogen (N) retention and reduce N excretion. However, feeds with excess dietary energy can reduce feed intake (FI) and result in reduced growth and efficiency. Therefore, determination of optimum digestible protein (DP) and DP/DE ratios is essential for sustainable production of this species. The objective of this investigation was to evaluate a wide range of DP/DE ratios to determine the optimal DP/DE ratio and minimal DP levels for maximizing growth, efficiency, and N gain of juvenile Florida pompano reared in seawater recirculating systems.

Materials and Methods

Growth Trial

Florida pompano broodstock were spawned at the United States Department of Agriculture (USDA), Agricultural Research Service's Center for Reproduction and Larviculture at Harbor Branch Oceanographic Institution, Fort Pierce, Florida, USA. Postlarval juveniles were reared at 28 C and 30 g/L salinity and fed a 55% CP and 14% CL commercial diet (EPAC-CW or IDL-CW; INVE Americas, Salt Lake City, UT, USA).

The experimental system was an 8750-L recirculating system with sand, bead, cartridge and carbon filtration, and ultraviolet light sterilization. The system was maintained at 28 C and received weekly water exchanges, approximately 10% by volume. Forty-eight 100-L tanks with a nominal flow rate of 3 L/min served as experimental units. Temperature, dissolved oxygen, and salinity were measured twice daily. Once-daily measurements of total ammonia nitrogen and nitrite-nitrogen were made using a HACH® test kit and DR/890 Colorimeter (HACH Co., Loveland, CO, USA) and pH with an Accumet AR25 pH meter (Fisher Scientific, Suwannee, GA, USA). A natural light cycle was used, approximating 11 h light and 13 h dark.

A completely randomized design with four replicates was employed with 12 experimental diets serving as the fixed classification effect. Experimental diets were formulated (Table 1) with an array of five CP and five CL levels (Fig. 1) to provide linearly increasing estimated DP/DE ratios ranging from 18.9 to 26.8 mg/kJ. For purposes of formulation, DE values were estimated on apparent digestibility of 80% CP, 85% CL, and 50% nitrogen-free extract and gross energy (GE) values of 23.5, 39.5, and 17.2 kJ/g dry matter (DM), respectively (NRC 1993).

All dry ingredients were mixed in a liquid–solid V-mixer (Patterson-Kelly, East Stroudsburg, PA, USA). Ingredients were transferred to a Hobart mixer (Hobart Corp., Troy, OH, USA), where water and menhaden fish oil were added under constant mixing. Sipernat (Degussa Corp., Parsippany, NJ, USA) was added to enhance pellet stability. Diets were cold pressed through a 1-mm die, dried in a forced air convection oven at 60 C for 24 h, and stored at −20 C until fed.

At initiation of the experiment, 15 fish were collected and stored at −20 C for subsequent proximate analysis. Twelve fish each, mean weight 6.3 ± 0.50 g, were stocked into the experimental units and randomly assigned dietary treatments. Fish were hand-fed as much as they could consume in 5 min up to apparent satiation, taking care that no food was wasted and FI recorded. Fish were fed twice daily except on days weighed when they did not receive a morning ration. Fish were weighed at 2-wk intervals during the 10-wk study.

At termination of the study, fish were starved for 24 h and collectively weighed by experimental unit, and six fish from each were randomly collected and euthanized in 150 mg/L tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). Three fish were pooled and stored at −20 C for subsequent proximate analysis. The remaining three fish were individually weighed and dissected for collection of liver and intraperitoneal fat (IPF). Hepatosomatic index (HSI) was determined, and livers stored at −80 C for subsequent analysis.

Standard procedures were used for determining proximate components (AOAC 2000). Pooled whole-body samples were minced in a meat grinder and further homogenized using mortar and pestle. Two samples from each

TABLE 1. *Composition of experimental diets (g/kg DM) fed to juvenile Florida pompano,* Trachinotus carolinus.

Experimental diet	1	2	3	4	5	6	7	8	9	10	11	12
Estimated DP/DE (mg/kJ)	26.8	25.4	24.8	23.5	23.6	22.0	21.9	21.8	21.0	20.7	19.0	18.9
Ingredients												
Menhaden meal[1]	348.4	264.1	369.8	331.0	284.0	354.0	224.0	247.0	338.5	283.0	248.0	224.0
Soybean meal[2]	191.0	133.2	213.0	213.0	182.0	234.0	113.0	155.0	221.0	182.0	155.0	113.0
Corn gluten meal[2]	80.0	50.0	73.0	80.0	57.0	73.0	42.0	50.0	68.0	57.0	50.0	42.0
Porcine blood meal[2]	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dehydrated fish solubles[3]	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Shrimp meal[2]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Dextrin[4]	29.0	58.0	23.0	21.0	39.0	16.0	68.0	51.0	22.0	39.0	51.0	68.0
Menhaden oil[5]	18.0	26.0	56.0	78.8	64.0	117.0	70.0	87.0	139.0	124.0	147.0	130.0
Sipernat 50[6]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix[7]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin premix[8]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Lecithin[9]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay-C 35[10]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose[11]	137.1	272.2	68.7	79.7	177.5	9.5	286.5	213.5	15.0	118.5	152.5	226.5
Carboxymethyl cellulose[11]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Chromic oxide[11]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dietary composition												
Crude protein (g/kg DM)	484	377	502	480	420	499	326	382	483	413	366	342
Crude lipid (g/kg DM)	75	68	113	121	102	157	91	116	180	145	189	157
Ash (g/kg DM)	134	105	140	126	117	133	95	111	133	118	109	100
Nitrogen-free extract (g/kg DM)[12]	120	120	120	120	120	120	120	120	120	120	120	120
Gross energy (MJ/kg DM)	18.04	17.75	18.94	19.80	19.17	20.64	18.73	19.03	21.03	20.59	20.53	19.73
Empirically determined												
Digestible energy (MJ/kg DM)	10.16	8.04	12.78	12.58	11.50	15.92	7.63	9.03	15.41	12.99	11.94	11.02
Digestible protein (g/kg DM)	360	278	392	356	326	399	229	261	366	303	263	261
DP/DE (mg/kJ)	35.4	34.6	30.7	28.3	28.4	25.1	30.0	28.9	23.8	23.3	22.0	23.7

DM = dry matter; DP/DE = digestible protein to digestible energy.

[1] Special Select, Omega Protein, Inc., Houston, Texas, USA.

[2] Rangen Inc., Buhl, Idaho, USA.

[3] International Proteins Corp., Minneapolis, Minnesota, USA.

[4] MP Biomedicals, Solon, Ohio, USA.

[5] Alkali refined and stabilized with 500 ppm ethoxyquin, Omega Protein, Inc., Hammond, Louisiana, USA.

[6] Degussa Corp., Parsippany, New Jersey, USA.

[7] Mineral premix contained the following (g/kg premix): $CaHPO_4$, 350.0; $CaSO_4{\cdot}2H_2O$, 100.0; KH_2PO_4, 200.0; $MgSO_4{\cdot}7H_20$, 84.0; $FeSO_4{\cdot}7H_2O$, 16.0; $ZnSO_4{\cdot}7H_2O$, 3.0; $MnSO_4{\cdot}H_2O$, 2.0; $CuCl_2{\cdot}2H_2O$, 1.0; KF, 0.23; KI, 0.1; $NaMoO_4{\cdot}2H_2O$, 0.05; $CoCl_2{\cdot}6H_2O$, 0.02; and Na_2SeO_3, 0.01.

[8] Roche warm water fish vitamin premix (Roche Vitamins Inc., Parsippany, NJ, USA).

[9] USB, Cleveland, Ohio, USA.

[10] Roche Vitamins, Inc.

[11] Sigma-Aldrich, St. Louis, Missouri, USA.

[12] Nitrogen-free extract estimated on calculated values (NRC 1993).

homogenized pool were taken for moisture analysis and dried at 105 C for 24 h. Tissues remaining in the pooled samples were analyzed for CP, CL, ash, and GE. Nitrogen was determined by combustion (TruSpec N-Elemental Analyzer; Leco Corp., St. Joseph, MI, USA), and CP calculated as N × 6.25. Ash was determined following incineration at 600 C for 2 h. CL was determined gravimetrically following chloroform : methanol extraction (Bligh and Dyer 1959) in a Soxhlet apparatus. GE was determined by adiabatic bomb calorimetry (Parr 1266; Parr Instruments Co., Moline, IL, USA). Treatments were evaluated for weight gain (WG), average daily gain (ADG), FE, protein efficiency ratio (PER), energy retention (ER),

		Dietary Crude Protein (g/kg DM)				
		34	38	42	48	50
Dietary Lipid (g /kg DM)	6		25.4		26.8	
	10	21.9		23.6		24.8
	12		21.8		23.5	
	16	18.9		20.7		22.0
	18		19.0		21.0	

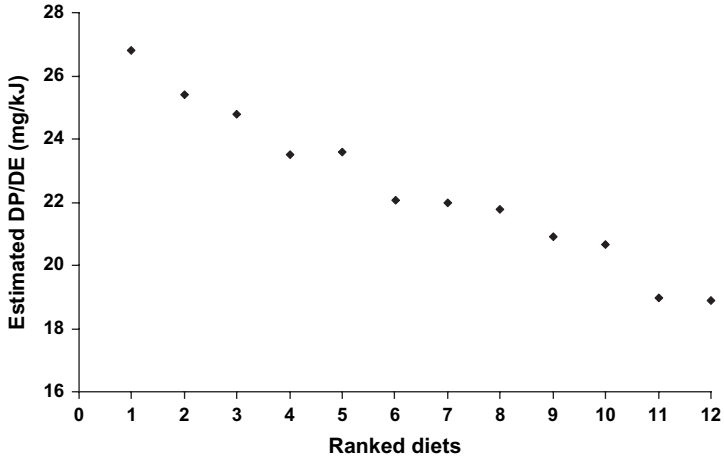

FIGURE 1. *Linearly decreasing estimated digestible protein to digestible energy ratios (DP/DE) resulting from the formulated crude protein and lipid array of dietary incorporation.*

thermal growth coefficient (TGC), and protein productive value (PPV).

Liver samples were lyophilized to a constant weight for determination of moisture. Hepatic protein and lipid were determined as above. Glycogen was determined by a modified phenol–sulfuric acid colorimetric assay (Lo et al. 1970) at a wavelength of 490 nm (DU640 Spectrophotometer; Beckman Coulter Inc., Fullerton, CA, USA).

Digestibility Trial

Siblings of fish used in the growth trial were used for determination of ADC for protein and energy for the experimental diets. Eighteen fish (approximately 100 g each) were stocked into each of 12 tanks on the same experimental system and randomly assigned one of the experimental diets. Each tank served as an experimental unit. Experimental conditions were as in the growth trial.

Fish were fed their assigned diets for a 6-d preliminary period for dietary acclimation. Fish were fed as in the growth trial. A preliminary experiment with similar-sized pompano and conditions used in the digestibility trial was conducted to determine intestinal transit time following a meal of a pigment (Cr_2O_3) containing feed after the method of Riche et al. (2004). First appearance of the pigment indicated intestinal transit was 3 h. Therefore, on Day 7, fish were fed in the morning and fecal samples collected 3–4 h later.

Fish were anesthetized with 80 mg/L MS-222, and samples obtained by gentle manual stripping of the lower intestine (Austreng 1978). Collected feces were pooled by experimental unit and dried at 105 C for 24 h. Fish were resuscitated and returned to their experimental tank. Dietary assignments were rerandomized, and the procedures repeated two more times. The three fecal collection periods served as replicates. No experimental units received the same diet twice and no mortalities occurred.

Dried fecal samples were ground and homogenized by mortar and pestle. A 100 mg aliquot was wet ashed by the method of Divakaran et al. (2002). Chromium (Cr) analysis was conducted

by inductively coupled plasma spectroscopy with an Optima 2100-DV ICP-OES (PerkinElmer, Shelton, CT, USA). Remaining feces were analyzed for GE and CP as previously described. Apparent digestibility coefficients (ADC) for protein and energy were calculated according to Maynard and Loosli (1969) as follows:

$$\mathrm{ADC} = 100 - (\%\mathrm{Cr}_{(\mathrm{feed})} \times \%\mathrm{Nutrient}_{(\mathrm{feces})})/(\%\mathrm{Cr}_{(\mathrm{feces})} \times \%\mathrm{Nutrient}_{(\mathrm{feed})}) \times 100.$$

Statistical Analysis

A completely randomized design with diet as a fixed factor effect was employed for the growth trial. Parametric assumptions were tested using Levene's test (homogeneity of variances) and Wilks–Shapiro test (normal distributions). Final body weight, efficiency parameters, whole-body composition, and tissue response variables were analyzed as a one-way ANOVA using the general linear model of SAS (SAS statistical software, version 8; Cary, NC, USA). All data were normally distributed. When marginal homogeneity of variance was detected, means were tested with Welch's ANOVA test for heterogeneous variances. When significant differences were detected, means were separated using Student–Newman–Keuls multiple range test. Significant differences were reported at the $P < 0.05$ level unless otherwise indicated. Means of variables analyzed with unequal cell sizes ($n = 3$) because of insufficient tissue were tested using least squares estimates of marginal means followed by a Tukey–Kramer multiple comparison adjustment to control for the experimentwise error rate.

Multiple regression analysis was conducted on gain, efficiency, and tissue composition parameters. Stepwise multiple regression analyses were run with response variables as the dependent variables and digestible energy intake (DEI), digestible protein intake (DPI), and DP/DE ratio as the independent regressors. Relationships were considered significant at the $P < 0.05$ level.

A randomized complete block design with blocking on time was employed for the digestibility trial. Diet served as the fixed classification effect, with each of the three blocks serving as replicates. ADC were analyzed using a mixed-effects model with the mixed procedure of SAS (SAS statistical software, version 8). Variance components were estimated using the restricted maximum likelihood method. When differences were detected, means were separated using the least-squares means method following the Tukey–Kramer adjustment to control for the experimentwise error rate (Littell et al. 2006). Significant differences were reported at the $P < 0.05$ level unless otherwise indicated.

Results

Water quality and water chemistry parameters during the growth trial are presented in Table 2. Values were maintained within suitable parameters for Florida pompano throughout the trial (Watanabe 1995; Weirich and Riche 2006). No mortalities occurred during the experiment.

Mean apparent CP digestibility (ACPD) coefficients and apparent energy digestibility (AED) coefficients determined in the digestibility trial are presented in Table 3. No difference was detected in ACPD coefficients. Conversely, significant differences were observed in AED coefficients, which ranged from 40.8 to 77.1%. Multiple regression with dietary ingredients having variable levels of incorporation as regressors indicated that dietary cellulose had a significant inverse effect on AED ($P < 0.001$; $R^2 = 0.92$). No other dietary ingredients were detected as having a significant effect on AED. ADC were applied to dietary protein and energy to determine DP and DE (Table 1). The empirically derived ratios were higher than the estimated ratios. The derived ratios were used for subsequent discussion and diet identification in Tables 4 and 5.

Mean daily intake (MDI) ranged from 0.60 to 0.94 g/fish/d (Table 4). Fish fed Diets 6 and 9 with DP/DE of 25.1 and 23.8 mg/kJ, respectively, consumed significantly more feed than those fed the remaining diets. Conversely, fish fed Diets 2 and 7 with DP/DE of 34.6 and 30.0 mg/kJ, respectively, consumed significantly less feed than the others. Mean DEI ranged from 4.2 to 13.0 kJ/fish/d and followed

TABLE 2. *Water chemistry and water quality parameters during the 10-wk growth trial with juvenile Florida pompano,* Trachinotus carolinus.

Parameter	Mean (±SD)
Temperature (C)	27.9 (0.51)
Salinity (g/L)	30.6 (1.03)
Dissolved oxygen (mg/L)	6.0 (0.45)
pH	7.8 (0.25)
Alkalinity (mg/L as $CaCO_3$)	108 (23.9)
Total ammonia nitrogen (mg/L)	0.10 (0.05)
Nitrite-N (mg/L)	0.37 (0.24)

a similar trend as MDI. Mean DPI ranged from 0.13 to 0.32 g/fish/d, with significantly higher DPI associated with higher DP. Moreover, significant differences in DPI were detected between diets at similar DP levels, with diets having higher DE resulting in higher DPI.

Final weight and other growth parameters followed similar trends, with significantly better gains in Diets 6 and 9, which had the highest DE levels (Table 1). Diets 6 and 9 resulted in WGs of 675 and 700%, respectively. Conversely, the lowest gains were observed in fish fed Diets 2 and 7 with the lowest DE levels. These diets resulted in gains of 246 and 259%, respectively. ADG was the same at 366 or 399 g DP/kg DM when DE was greater than 15 MJ/kg DM (Fig. 2).

TABLE 3. *Mean (*n = *3) apparent digestibility coefficients for crude protein and energy in juvenile Florida pompano,* Trachinotus carolinus*, fed diets with varying protein to energy ratios.*[1]

Experimental diet (estimated DP/DE)[2]	Crude protein (%)	Energy (%)
1 (26.8)	74.5	56.3^{bcd}
2 (25.4)	73.6	45.3^{cd}
3 (24.8)	78.1	67.4^{ab}
4 (23.5)	74.1	63.6^{ab}
5 (23.6)	77.7	60.0^{bc}
6 (22.0)	79.9	77.1^{a}
7 (21.9)	70.2	40.8^{e}
8 (21.8)	68.2	47.5^{cde}
9 (21.0)	75.8	73.3^{a}
10 (20.7)	73.4	63.1^{ab}
11 (19.0)	71.7	58.1^{bcd}
12 (18.9)	76.3	55.9^{bcde}
Pooled SE	2.50	2.44

[1] Values within a column with different superscripts are significantly different ($P < 0.05$).

[2] Digestible protein to digestible energy (DP/DE) ratio (mg/kJ).

Similar to FI and growth, FE was highest in Diets 6 (0.77) and 9 (0.75) and lowest in Diets 7 (0.43) and 2 (0.40). Regression analysis suggested that increased FE was principally attributed to DPI ($P < 0.001$; partial $R^2 = 0.88$).

PER in fish fed Diet 11 was 2.26 and significantly higher than in those fed all other diets except 10, which were the two diets with the lowest DP/DE. The lowest PER values of 1.69 and 1.44 occurred in fish fed Diets 1 and 2, which also had the highest DP/DE ratios of 35.4 and 34.6 mg/kJ, respectively. Multiple regression suggested that PER was independent of DPI but related to DP/DE ($P < 0.001$; $R^2 = 0.63$). The PPV followed a similar trend to PER; however, unlike with PER, PPV in fish fed Diet 11 was significantly higher than in those fed Diet 10. The PPV was highly correlated with PER ($R^2 = 0.96$). ER ranged from 36.9 to 48.9%, with no readily apparent trends.

No discernible IPF was detected for any treatments. Whole-body and liver compositions are presented in Table 5. Whole-body CP was lowest in fish fed Diet 12, which was significantly lower than in those fed Diets 3, 4, and 6. Whole-body CP was also significantly higher in fish fed Diet 3 than in those fed Diets 5 and 10. Whole-body lipid was significantly higher in fish fed Diets 9 and 11 than in those fed Diets 1, 2, and 3, which had the highest DP/DE ratios, and those fed Diets 7 and 12, which had the lowest DP levels, had lower lipid than in those fed Diet 9. Moisture and lipid were inversely related, $R^2 = 0.91$.

Hepatic moisture tended to decrease with DP/DE, and only Diets 11 (22.0 mg/kJ) and 12 (23.7 mg/kJ) had significantly less moisture than Diet 1, with the highest DP/DE at 35.4 mg/kJ. With the exception of fish fed Diet 9, hepatic protein followed the same trend as hepatic moisture. However, only fish fed Diet 12 contained less hepatic protein than the other treatments. Conversely, the diets with the lowest DP/DE had the highest concentration of hepatic lipid, but only Diets 1, 6, and 8 were significantly lower than Diet 11. Similarly, fish fed Diets 11 and 12 exhibited the highest concentrations of

TABLE 4. *Mean gain, intake, and selected efficiency parameters in juvenile Florida pompano,* Trachinotus carolinus, *fed diets varying in DP/DE ratios.*[1]

Diet	1	2	3	4	5	6	7	8	9	10	11	12	Pooled
DP/DE (mg/kJ)	35.4	34.6	30.7	28.3	28.4	25.1	30.0	28.9	23.8	23.3	22.0	23.7	SE
Growth													
WG[2]	445^{cd}	246^{e}	596^{b}	584^{b}	399^{d}	675^{a}	259^{e}	364^{d}	700^{a}	515^{bc}	440^{cd}	357^{d}	12.6
ADG[3]	0.33^{c}	0.18^{e}	0.42^{b}	0.43^{b}	0.29^{d}	0.51^{a}	0.19^{e}	0.28^{d}	0.49^{a}	0.38^{b}	0.33^{c}	0.26^{d}	0.01
TGC[4]	1.51^{d}	0.78^{f}	1.89^{bc}	1.94^{b}	1.30^{de}	2.32^{a}	0.88^{f}	1.27^{de}	2.31^{a}	1.71^{c}	1.47^{d}	1.14^{e}	0.07
Intake													
MDI[5]	0.76^{cd}	0.60^{e}	0.83^{b}	0.85^{b}	0.71^{d}	0.92^{a}	0.63^{e}	0.72^{cd}	0.94^{a}	0.79^{bc}	0.76^{cd}	0.69^{d}	0.02
DEI[6]	6.81^{d}	4.24^{f}	9.36^{b}	9.43^{b}	7.26^{d}	12.96^{a}	4.25^{f}	5.78^{e}	12.84^{a}	9.08^{b}	8.00^{c}	6.71^{d}	0.20
DPI[7]	0.24^{e}	0.15^{h}	0.29^{c}	0.27^{d}	0.21^{f}	0.32^{a}	0.13^{i}	0.17^{g}	0.31^{b}	0.21^{f}	0.18^{g}	0.16^{gh}	0.01
Efficiency													
FE[8]	0.61^{c}	0.40^{g}	0.70^{b}	0.70^{b}	0.56^{de}	0.77^{a}	0.43^{g}	0.54^{ef}	0.75^{a}	0.66^{b}	0.59^{cd}	0.51^{f}	0.01
ER[9]	43.7^{abcd}	36.9^{e}	45.7^{ab}	46.7^{a}	38.8^{cde}	39.3^{bcde}	45.1^{abc}	48.9^{a}	42.9^{abcde}	42.8^{abcde}	43.2^{abcde}	37.9^{de}	1.5
PER[10]	1.69^{g}	1.44^{h}	1.78^{efg}	1.97^{cd}	1.71^{fg}	1.93^{cde}	1.86^{def}	2.06^{bc}	2.05^{bc}	2.18^{ab}	2.26^{a}	1.95^{cd}	0.04
PPV[11]	29.0^{de}	24.3^{f}	31.4^{cd}	34.1^{bc}	28.2^{e}	33.3^{bc}	31.8^{cd}	34.8^{b}	34.6^{b}	35.8^{b}	38.1^{a}	31.5^{cd}	0.74
Survival (%)	100	100	100	100	100	100	100	100	100	100	100	100	

FE = feed efficiency; DEI = digestible energy intake; DPI = digestible protein intake; TGC = thermal growth coefficient; PER = protein efficiency ratio; ER = energy retention; PPV = protein productive value; ADG = average daily gain; WG = weight gain; MDI = mean daily intake; DP/DE = digestible protein to digestible energy.

[1] Mean values ($n = 4$) across a row with different superscripts are significantly different ($P < 0.05$).

[2] WG (% increase).

[3] ADG (g/d).

[4] $\text{TGC} = [(\text{weight}^{0.33}_{(\text{final})} - \text{weight}^{0.33}_{(\text{initial})})/\Sigma\text{T}] \times 100$, where ΣT = sum of degree days.

[5] MDI (g/fish/d).

[6] DEI (kJ/fish/d).

[7] DPI (g/fish/d).

[8] $\text{FE} = (\text{weight}_{(\text{final})} - \text{weight}_{(\text{initial})})/\text{total feed intake}$.

[9] $\text{ER}\ (\%) = [(\text{weight}_{(\text{final})} \times \text{energy}_{(\text{final})}) - (\text{weight}_{(\text{initial})} \times \text{energy}_{(\text{initial})})/\text{DEI}] \times 100$.

[10] $\text{PER} = (\text{weight}_{(\text{final})} - \text{weight}_{(\text{initial})})/\text{DPI}$.

[11] $\text{PPV}\ (\%) = [(\text{weight}_{(\text{final})} \times \text{protein}_{(\text{final})}) - (\text{weight}_{(\text{initial})} \times \text{protein}_{(\text{initial})})/\text{DPI}] \times 100$.

TABLE 5. *Whole-body (fish) and liver compositions of juvenile Florida pompano,* Trachinotus carolinus*, fed experimental diets with varying DP/DE ratios.*[1]

Diet	1	2	3	4	5	6	7	8	9	10	11	12	Pooled
DP/DE (mg/kJ)	35.4	34.6	30.7	28.3	28.4	25.1	30.0	28.9	23.8	23.3	22.0	23.7	SE
Fish[2]													
Moisture	71.1^{a}	71.4^{a}	68.9^{bc}	67.6^{bc}	69.0^{bc}	68.5^{bc}	69.4^{b}	68.6^{bc}	67.1^{c}	68.5^{bc}	67.2^{bc}	68.3^{bc}	0.46
CP	16.7^{abc}	16.3^{abc}	17.2^{a}	17.0^{ab}	16.1^{bc}	16.9^{ab}	16.4^{abc}	16.5^{abc}	16.6^{abc}	16.2^{bc}	16.5^{abc}	15.9^{c}	0.21
CL	9.9^{e}	10.5^{de}	12.0^{cde}	13.7^{abc}	12.5^{abcd}	13.1^{abc}	12.2^{bcd}	13.2^{abc}	14.7^{a}	13.2^{abc}	14.5^{ab}	12.2^{bcd}	0.53
Ash	3.3^{b}	3.6^{ab}	3.2^{b}	3.2^{b}	3.4^{ab}	3.3^{b}	3.6^{ab}	3.4^{ab}	3.1^{b}	3.3^{b}	3.5^{ab}	3.8^{a}	0.11
Energy	24.5^{c}	24.6^{c}	25.8^{a}	24.9^{c}	24.6^{c}	24.9^{bc}	24.6^{c}	24.8^{c}	25.8^{a}	25.6^{ab}	25.0^{bc}	24.5^{c}	0.15
Liver[3]													
Moisture	74.0^{a}	73.2^{ab}	72.8^{ab}	69.9^{abc}	71.3^{abc}	73.0^{ab}	72.4^{ab}	73.4^{ab}	72.5^{ab}	70.0^{abc}	68.3^{c}	69.7^{bc}	0.87
CP	14.7^{a}	14.5^{a}	14.6^{a}	14.1^{a}	14.4^{a}	13.8^{a}	13.4^{a}	13.5^{a}	14.3^{a}	13.4^{a}	13.1^{a}	11.8^{b}	0.41
CL	9.3^{b}	12.4^{ab}	11.8^{ab}	12.7^{ab}	11.8^{ab}	9.2^{b}	12.7^{ab}	9.7^{b}	12.2^{ab}	13.8^{ab}	15.1^{a}	13.5^{ab}	0.98
Glycogen	5.2^{ab}	4.7^{b}	6.0^{ab}	7.8^{ab}	5.9^{ab}	7.7^{ab}	7.7^{ab}	6.8^{ab}	6.4^{ab}	6.9^{ab}	9.0^{ab}	12.0^{a}	1.30
HSI[4]	1.15^{ab}	1.02^{ab}	0.93^{b}	0.90^{b}	1.18^{ab}	1.04^{ab}	1.00^{ab}	1.05^{ab}	1.17^{ab}	1.17^{ab}	1.25^{ab}	1.41^{a}	0.09

CP = crude protein; CL = crude lipid; HSI = hepatosomatic index; DP/DE = digestible protein to digestible energy.
[1] Mean values ($n = 4$) across a row with different superscripts are significantly different ($P < 0.05$).
[2] Moisture, CP, CL, and ash are % wet weight; energy is MJ/kg fish.
[3] Reported as % fresh weight basis.
[4] HSI (%) = (wet liver weight/total wet body weight) × 100.

hepatic glycogen and had the highest HSI, but HSI was only significantly different between Diets 2 and 12. Multiple regression suggested that differences in HSI could not be explained by hepatic lipid, protein, or glycogen.

Discussion

ADC for protein and energy were substantially lower than reported for some other marine species (Peres and Oliva-Teles 1999; Santinha et al. 1999; Sá et al. 2006) but similar to those previously reported for Florida pompano (Williams et al. 1985). Possible explanations include ingredient differences, fecal collection method, and gastric evacuation rates. In the current study, a multi-ingredient practical-type feed formulation was used. In addition, manual stripping of the intestinal tract was used instead of fecal settling columns. The former may potentially underestimate apparent digestibility

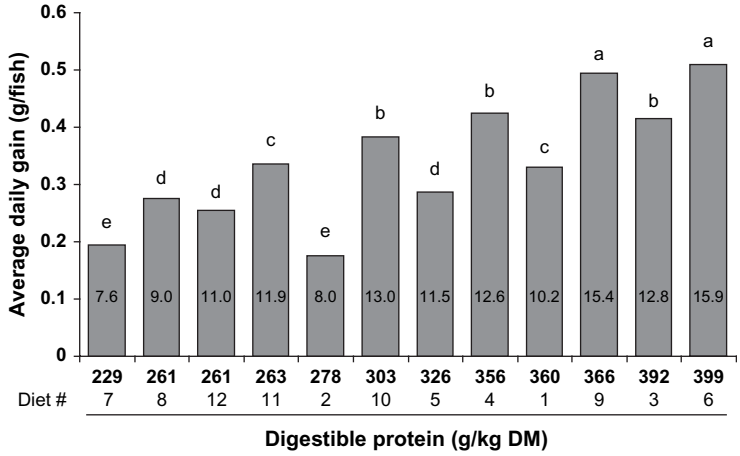

FIGURE 2. *Average daily gain as a function of digestible protein (g/kg DM) and digestible energy (MJ/kg DM; values inside bars) in juvenile Florida pompano,* Trachinotus carolinus*, fed diets with varying digestible protein and energy ratios. DM = dry matter.*

because of incomplete digestion, whereas the latter may overestimate apparent digestibility because of leaching of nutrients. Moreover, the preliminary investigation to determine intestinal transit time revealed that the intestinal transit time in Florida pompano at 28 C was 3 h, which was in accordance with Williams et al. (1985). This short transit may result in limited enzymatic contact time attenuating digestion and absorption of nutrients, possibly causing the poor feed conversions previously reported for Florida pompano (Tatum 1973; Williams et al. 1985; Lazo et al. 1998; Weirich et al. 2006).

It has been well demonstrated that excess dietary energy can decrease consumption, resulting in reduced growth and conversion. In the current study, MDI (g/fish/d) significantly increased ($P < 0.001$) as DE increased (Fig. 3); however, when standardized to body weight (mg/g fish/d), consumption was higher ($P < 0.001$) in fish fed the lower energy diets. This suggests that fish fed the low-energy diets increased their consumption to meet their energy needs. Consequently, DE ($R^2 = 0.86$) and DEI ($R^2 = 0.94$) were strongly correlated with growth measured as ADG. That consumption (g/fish/d) increased with increasing DE and decreased as a function of weight (mg/g fish/d) suggests that higher MDI was related to faster growth and growth was limited by DEI. Multiple regression analysis suggested that cellulose significantly decreased energy digestibility and available dietary energy as observed in rainbow trout, *Oncorhynchus mykiss* (Bromley and Adkins 1984). Similarly, DE significantly increased with increasing dietary energy ($P < 0.012$), but this was likely an artifact of increasing dietary energy with menhaden oil at the expense of cellulose. Moreover, diets high in cellulose resulted in increased consumption, likely to maintain constant energy intake as a compensation mechanism for nutrient dilution (Bromley and Adkins 1984).

ADG increased as a function of both DP and DE (Fig. 2). At 11.5 MJ/kg diet, there was insufficient DE to support maximum growth as there was no difference in growth at this energy level whether the diet contained 261 or 326 g DP/kg DM (Diets 12 and 5), representing 23.7 and 28.4 mg/kJ, respectively. This suggests that protein in the higher DP diet was being catabolized for energy. In support of this conclusion, increasing the DE from 9.0 to 11.9 MJ/kg significantly increased growth in fish fed 263 g DP/kg DM (Diet 11) relative to those fed 326 g DP/kg DM (Diet 5). Similarly, differences in growth were not observed when holding DE relatively constant at 12.6–13.0 MJ/kg DM (Diets 3, 4, and 10) and increasing DP from 303 to 392 g/kg DM, representing 23.3–30.7 mg/kJ, suggesting DE of 13.0 MJ/kg DM was also insufficient to attain maximum growth. Conversely, holding DP relatively constant at 356–366 g DP/kg diet (Diets 1, 4, and 9) and increasing DE from 10.2 to 15.4 MJ/kg DM resulted in a stepwise increase in growth, supporting the hypothesis that juvenile Florida pompano require high-energy diets to meet their maximum growth potential.

Maximum growth was attained at DE levels greater than 15 MJ/kg DM, which approximated a DP/DE of 24 mg/kJ. Although Diet 6 containing 399 g DP/kg DM contained more DE than Diet 9 containing 366 g DP/kg DM, no further increases in growth were observed. Moreover, growth increased stepwise with increasing DP in all the diets containing a DP/DE ratio of 25 mg/kJ or less until the diet contained 366 g DP/kg DM after which ADG reached a plateau. This would indicate that 366 g DP/kg DM was sufficient for attaining maximum growth. Excess protein again was likely catabolized. Lazo et al. (1998) reported that the dietary CP requirement for Florida pompano was no less than 45%; assuming an apparent protein digestibility of 82%, this would be similar to the 366 g DP/kg DM reported here.

FE ranged from 0.40 to 0.77, which bracketed other FE values reported for Florida pompano (Williams et al. 1985; Lazo et al. 1998), and similar to the mean value (0.73) reported for similar-sized Florida pompano (Weirich et al. 2006). The FE values similar to or lower than those reported by Williams et al. (1985) were low in DP, and those higher had high DP and/or substantially higher DE. This would suggest that the poor FE reported by Williams et al. (1985) was likely a result of overestimating DP and insufficient DE.

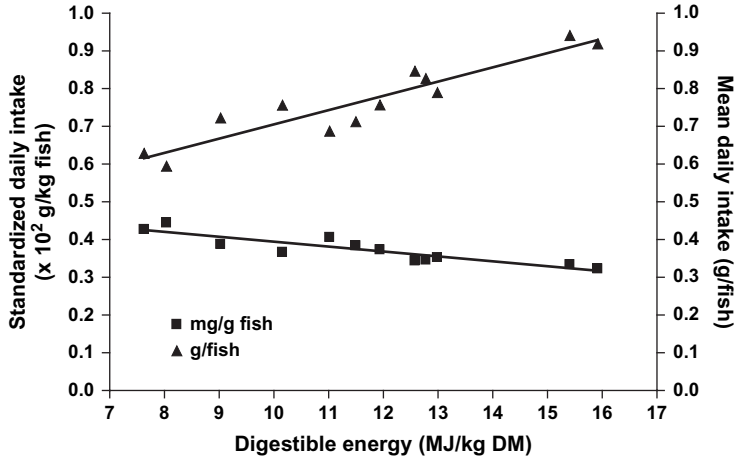

FIGURE 3. *Mean daily intake per individual (g/fish,* $y = 0.038x - 8.6$; $R^2 = 0.87$*) and mean daily intake following standardization to wet body weight (mg/g fish,* $y = -0.013x + 40.4$; $R^2 = 0.81$*) in juvenile Florida pompano,* Trachinotus carolinus*, as a function of digestible energy.*

Whole-body protein values were consistent with other species, and although statistical differences exist, the reason is not readily apparent as protein unlike lipid is relatively constant across species and independent of size (Lupatsch et al. 2003). Whole-body lipid levels ranged from 9.9 to 14.7%, and statistical differences were centered around fish fed low-DP/DE diets and the highest DP/DE diets. Williams et al. (1985) suggested that differences in body lipid were an indicator of excess dietary energy in Florida pompano when fed diets containing 36.8 kJ/g DP (27 mg/kJ) relative to 28 and 34 kJ/g DP (32 and 29 mg/kJ, respectively). However, as stated earlier, their DP was likely overestimated, resulting in a lower DP/DE ratio than reported. Weirich et al. (2006) reported whole-body lipid levels of 13.1–14.7%; however, a direct comparison is difficult. Although high dietary lipid can increase carcass lipid (Tibaldi et al. 1996; Company et al. 1999; Peres and Oliva-Teles 1999; de Borba et al. 2003; Williams et al. 2003), no clear trend between whole-body lipid and dietary lipid was evident.

Surplus dietary energy has also been shown to result in accumulation of mesenteric fat (Craig et al. 1999; Peres and Oliva-Teles 1999; Martino et al. 2002; Du et al. 2005). However, in the current study, no discernible mesenteric fat was detected in any treatment, consistent with Weirich et al. (2006). Similarly, excess dietary energy can lead to hepatic pathologies. Fish fed Diets 10, 11, and 12 (DP/DE of 23.3, 22.0, and 23.7 mg/kJ, respectively) exhibited the lowest hepatic moisture and protein and highest lipid, suggesting that a DP/DE lower than 24 mg/kJ may contain excessive energy when DP is less than 360 g/kg DM. These diets also contained among the highest dietary lipid, which can lead to hepatic steatosis (Spisni et al. 1998). However, in marine species, higher hepatic lipid does not necessarily represent a pathological disorder but may indicate a well-fed state (Caballero et al. 1999). Further research and histological examination are warranted to determine if this deposition represents energy storage or onset of a pathological process.

The objective of culturing fish for consumption is production of protein; therefore, efficiency of converting dietary protein into protein accretion should be the principal determinant in ascribing optimal protein and energy requirements. The PPV ranged from 24.3 to 38.1%, with the Diet 2 resulting in 24.3% low in protein and energy, and the Diet 11 resulting in 38.1% low in protein and high in energy. When sufficient energy is available, low-protein diets are generally more efficient in terms of N retention (Lupatsch et al. 2001). In Asian sea bass, *Lates calcarifer*, and gilthead sea bream,

Sparus aurata, apparent protein retention increased with dietary energy and lower protein levels (Catacutan and Coloso 1995; Lupatsch et al. 2001). However, regression analyses indicated no relationship between PPV and dietary or DP ($P = 0.936$ and $P = 0.914$, respectively). However, low dietary protein was efficiently used for protein synthesis; provisioning sufficient dietary energy was available. PER and PPV were highly and significantly correlated ($P < 0.001$; $R^2 = 0.96$), indicating that WG associated with N intake was almost exclusively in the form of protein accretion.

A graph of N gain (g/fish) as a function of DP (g/kg DM) exhibits a similar trend as ADG and DP, where accretion is a function of both DP and DE (Fig. 4). Similar to Lupatsch et al. (2001), increasing energy at constant DP resulted in improved protein utilization. This is most apparent with the fish fed the diets with 356 and 366 g DP/kg DM and the diets with 392 and 399 g DP/kg DM. There was no increase in protein accretion when DP was 356 or 392 g/kg DM and when DE was 12.6 and 12.8 kJ/g DM. Similarly, there was no increase in protein accretion when DP was 366 or 399 g/kg DM and when DE was 15.4 and 15.9 kJ/g DM. This would suggest a minimum DP requirement for juvenile Florida pompano of approximately 360 g/kg DM.

Based on growth and efficiency, optimal DP/DE approximates 24 mg/kJ similar to 25.9 mg/kJ in Japanese sea bass, *Lateolabrax japonicus* (Ai et al. 2004), but lower than 30.6 mg/kJ in Asian sea bass (Catacutan and Coloso 1995) and 27.5–29.5 mg/kJ in mutton snapper, *Lutjanus analis* (Watanabe et al. 2001). However, these investigators used physiological fuel values and dietary protein, not experimentally derived DP and energy. Recalculating the optimal ratio on this basis would increase the DP/DE for Florida pompano to 31.1 mg/kJ, underscoring the importance of determining DP and energy values for feed ingredients.

In summary, juvenile Florida pompano require high-energy diets to meet their metabolic and growth demands. Minimum DP to maximize growth and N gain is between 356 and 366 g/kg DM. Furthermore, DE to attain maximum growth is 15.4 MJ/kg DM, resulting in an optimum DP/DE ratio between 23.8 and 25.1 mg/kJ. Actual DP and DE values of feed ingredients should be determined for juvenile Florida pompano as nutrients appear to be less digestible than in some other species, likely because of a short intestinal transit time. Although Florida pompano do not store excess energy as mesenteric fat, they do maintain high hepatic lipid concentrations. Further research is warranted to determine if this condition represents onset of

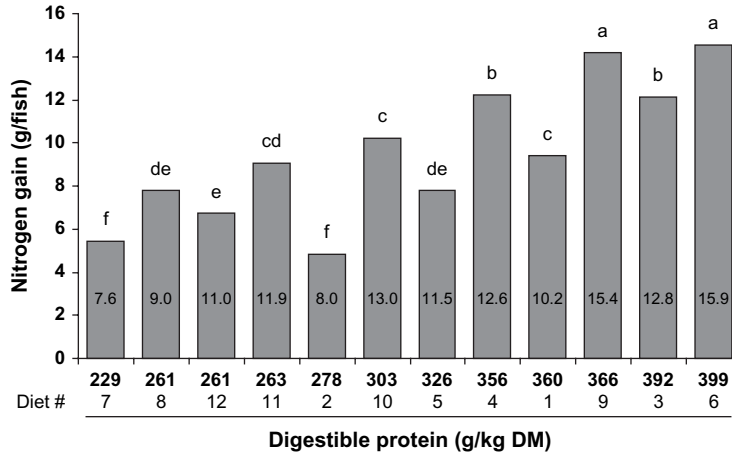

FIGURE 4. *Nitrogen gain as a function of digestible protein (g/kg DM) and digestible energy (MJ/kg DM; values inside bars) in juvenile Florida pompano,* Trachinotus carolinus*, fed diets with varying digestible protein and energy ratios. DM = dry matter.*

a pathological syndrome or energy storage indicative of a well-fed state.

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